# Dominulin A and B: Two New Antibacterial Peptides Identified on the Cuticle and in the Venom of the Social Paper Wasp *Polistes dominulus* Using MALDI-TOF, MALDI-TOF/TOF, and ESI-Ion Trap

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Two new antibacterial peptides, denominated as Dominulin A and B, have been found on the cuticle and in the venom of females of the social paper wasp *Polistes dominulus*. The amino acidic sequence of the two peptides, determined by mass spectrometry, is INWKKIAE VGGKIL SSL for Dominulin A (MW = 1854 Da) and INWKKIAEIGKQVL SAL (MW = 1909 Da) for Dominulin B. Their presence on the cuticle was confirmed using MALDI-TOF by means of micro-extractions and direct analyses on body parts. The presence in the venom and the primary structure of the dominulins suggest their classification in the mastoparans, a class of peptides found in the venom of other *Aculeate hymenoptera*. Their antimicrobial action against Gram+ and Gram− bacteria fits in the range of the best natural antimicrobial peptides. Dominulins can represent an important defense of the colony of *Polistes dominulus* against microbial pathogens. (J Am Soc Mass Spectrom 2006, 17, 376–383) © 2006 American Society for Mass Spectrometry

Colonies of social insects (termites, ants, and many species of wasps and bees) may consist of thousands of insects, and are therefore potentially prone to infections by pathogenic microorganisms [1]; moreover their vulnerability to pathogens is often enhanced by the reduced genetic variability of their members [2]. The first defense of the colony against spreading of infectious diseases is represented by a set of hygienic behaviors (such as the elimination of corpses and organic wastes and the removal of infected insects from the nest), and by the use of fungicides and bactericides which may be produced by the colony members or be collected from plants [1]. As a further protection, the immune system of insects responds to microorganisms penetrating the body by releasing antimicrobial and antifungal peptides and proteins into the hemolymph [3–6]. Although the fat body is clearly the main source of the inducible antipathogenic peptides in insects [4], epidermis, including epithelial cells underlying cuticle, has also been demonstrated to produce antibacterial and antifungal peptides in response to local infections [7, 8, and quotations reported therein]. Additionally, some of these peptides have also been found to be constitutively expressed in the female reproductive apparatus of some dipterans [8 and quotations reported therein]. Many antimicrobial secretions of insect origin have been reported, and several have been studied in social insects, and in particular in the honeybees. In ants, the metapleural gland produces secretions with antiseptic and antifungal activity [9 and references therein]. In leaf cutter ants, these secretions protect the ants themselves and their clonal mutualistic fungus [2].

For social wasps, it is known that larval saliva contains antimicrobial agents [10, 11], while an old

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paper by Pavan [12] reports antimicrobial activity of body ethanol extracts of the hornet *Vespa crabro*. Venom antimicrobial activity has already been reported in ants [13–15], and social wasps [16–20]. In general, however, the antiseptic exocrine secretions described so far are generally of non-proteic nature [1, 21 and literature cited therein].

The chemistry of the most external layer of the insect cuticle, the epicuticle, is well known for several insect species. The epicuticle is covered by complex mixtures of lipids [22 and references reported therein], which defend insects against dehydration and probably also play some role against microorganisms [23, 24, 25]. However, to the best of our knowledge, antimicrobial peptides have never been reported on the epicuticle, neither for solitary nor for social insects. To demonstrate a possible antimicrobial activity of the compounds found in this epicuticular layer, we performed microbiological tests and report here about the presence of two new antibacteric peptides on the surface of the female cuticle in the social wasp *Polistes dominulus*. We found these molecules also in the venom and we named them Dominulin A and Dominulin B. Their sequences, determined by MALDI-TOF/TOF experiments and confirmed by biochemical methods, were compared with those of peptides already reported for the venom of other social wasps. Their origin and social function is discussed after a survey of their presence in other components of the colonies (larvae, pupae, nest paper), and in different body parts of female insect.

## Materials and Methods

### Chemicals

Methanol, acetonitrile, and *n*-pentane were of chromatography grade and purchased by Riedel de Haen (Sigma Aldrich Italia, Milan, Italy). Purified and deionized water was prepared using a Milli-Q system (Millipore, Bedford, MA). Formic acid and trifluoroacetic acid (TFA) was purchased from Fluka (Sigma Aldrich Italia). The α-cyano 4-hydroxycinnamic acid was obtained from Bruker Daltonics (Bremen, Germany). The matrix for MALDI-TOF experiments was a mixture of saturated solution of α-ciano 4-hydroxycinnamic acid in acetonitrile and 0.1% TFA in water (1:2, vol:vol). Unless stated otherwise, all other reagents were of analytical grade and used as supplied.

### Biological Subjects

*Studied species.* *Polistes dominulus* is a common European species of paper wasp, which is now very common also in the United States, after it was accidentally imported almost 20 years ago. It has an annual colony cycle, unenveloped nests composed of a single comb, and colonies usually inhabited by up to 200 adults at the seasonal peak (usually in July) [26].

*Collection of insects for analyses.* Colonies of *Polistes dominulus* were collected in the countryside around Florence, Tuscany, Central Italy, during May and June 2002–2004. Colonies were stored in a freezer at −20 °C or reared for a while in the laboratory in glass cages (15 × 15 × 15 cm) with water, sugar, and fly maggots *ad libitum*. The external cuticular layer of single adult individuals were washed with solvents (methanol, *n*-pentane) to obtain extracts to be preliminarily tested for antimicrobial activity. Groups of females were used to collect large quantities of methanol extracts for RP-HPLC purification of the active substances. Single adult wasps, both male and female, eggs, larvae, pupae, and pieces of nest paper were used for the survey of particular peptides using MALDI-TOF.

To avoid transfer of substances between adult members of the colony, we also analyzed wasps (both males and females), previously artificially extracted from their operculated cells and allowed to complete their pupal development in plastic vials far from their nest. These analyses were performed three days after the emergence of the wasps from the artificial vials.

### Microbiological Assays

We assayed microbial growth inhibition by the agar diffusion method and determination of minimal inhibitory concentration (MIC).

*Agar diffusion tests.* The agar diffusion test was performed with the *Bacillus subtilis* strain ATCC 6633 on Nutrient Agar (Oxoid, Basingstoke, UK) as previously described [11]. Penicillin G, used as standard, was spotted on a plate surface as 1 μl solution (12.5 μg/ml). Substances to be tested for antimicrobial activity were spotted on a plate surface as 5 μl solution. Pure solvents (in spots of 5 μl) were also tested in the same Petri dishes. Petri dishes were checked after one day of incubation at 37 °C. Antimicrobial activity was indicated by clear zones of growth inhibition on the plates. At the beginning we tested (8 trials) for their antimicrobial activity the extracts in methanol (2 ml) of a group of 10 female *P. dominulus* and the extract in pentane (2 ml) of another group of 10 females. Extracts had been dried in an air stream and then resuspended in 200 μl of the same solvent. Then, we tested with the same technique the methanol extracts (each of 250 μl) obtained from 10 single female *P. dominulus*. We used this test also to check the antibacterial activity of the peaks fractioned with RP-HPLC from methanol extracts of females.

*Determination of minimal inhibitory concentration of synthetic peptides (MIC).* The MIC was defined as the peptide minimal concentration that completely inhibited microbial growth by eye evaluation of turbidity. Bacteria used were *B. subtilis* ATCC 6633 as Gram+ and *Escherichia coli* JM109 as Gram− reference strains. They were grown in Nutrient Broth (Oxoid) and in PY Antibiotic Medium N. 3 (Oxoid) media, respectively.

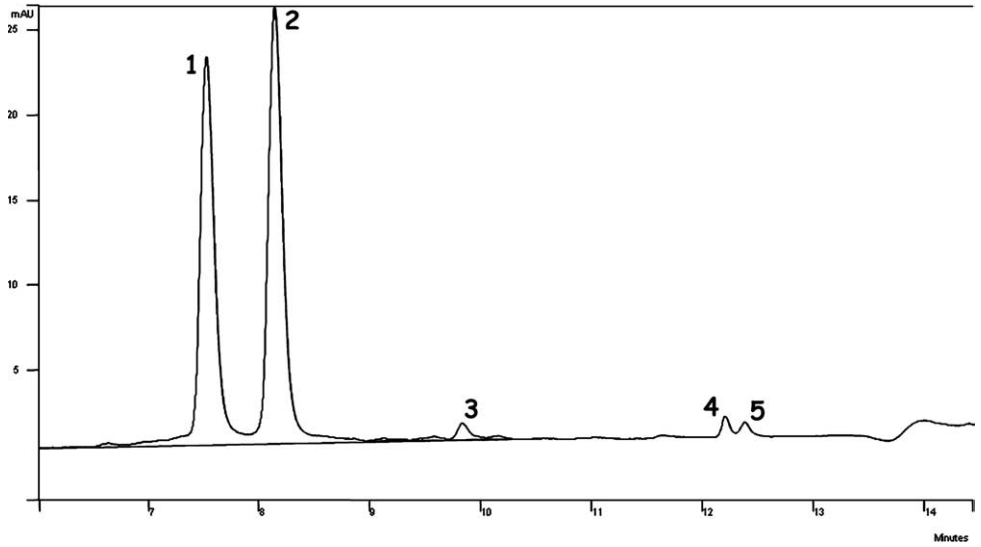


**Figure 1.** HPLC-UV analysis of methanol extract of the cuticle of female *P. dominulus* Peaks 1 and 2 correspond to Dominulin A and Dominulin B, respectively.

Overnight cultures were diluted in appropriate fresh medium; 50 $\mu$l of cell suspension containing 104 cells were inoculated in 900 $\mu$l of fresh medium and 50 $\mu$l of each peptide water solution (in a concentration range 0.1–100 $\mu$g/ml) in 12 ml glass tubes. Positive (50 $\mu$l of cells inoculated in 950 $\mu$l of medium) and negative (1000 $\mu$l of uninoculated medium) controls were prepared in each experiment. Cells were incubated at 37 °C with shaking at 110 rpm for 18 h. The experiments were done at least three times.

## Chemical Analyses

*Peptide fractionation.* Five groups of 100 insects were placed in methanol for 5 min in five 50 ml plastic tubes. Insects were removed and after centrifugation (10,000 *g*, room-temperature, 5 min) the solvent was recovered, reunited in one single tube, and completely evaporated. Extracts were then resuspended in 4 ml of a solution of water and acetonitrile (80:20 vol/vol containing 0.5% formic acid). HPLC-UV analyses of the extract showed the presence of 5 peaks (Figure 1). The two most abundant peptides were separated in a Series 200 (Perkin-Elmer, Boston, MA) HPLC system including an autosampler, a quaternary pump, and an UV-VIS detector coupled with a fraction collector Biologic BioFrac (BioRad, Hercules, CA). The RP-HPLC column was a Luna C8, 150 Å $\times$ 4.6 mm, 5 $\mu$m (Phenomenex, Torrance, CA), operating at a flow rate of 0.75 ml/min. The absorbance was monitored at 254 nm. Deionized water and acetonitrile, both containing 0.5% formic acid, were used as the eluents; the elution gradient program started at 20% acetonitrile, then to 50% acetonitrile in 3 min, and to 80% acetonitrile in 15 min. The same column, elution gradient program, and wavelength were used in all HPLC separations cited in the following paragraphs. The collected fractions were lyophilized and resuspended in a solution of water and acetonitrile (80:20 vol/vol containing 0.5% formic acid) for MS analysis.

*Mass spectrometry.* The methanolic extract solution and the solutions of the two principal peptides, prepared as above described, were analyzed by ESI-TOF, ESI-IT and MALDI- TOF instruments.

The solution obtained from the evaporated extract of groups of individuals was injected in an HPLC 9012 (Varian Italia, Leinì, Turin, Italy); after the UV detector, the eluate was split in a 1:4 ratio and about 200 $\mu$l/min were directed to the ESI interface of a Mariner TOF mass spectrometer (Applied Biosystems, Foster City, CA). The spray tip and nozzle potentials were set to 3.8 kV and 120 V, respectively. The positive ion ESI mass spectra were recorded. For the two principal peptides, these were characterized by the presence of intense doubly and triply charged species, indicating a molecular weight of 1854.1 Da and 1909.1 Da for Dominulin A and B, respectively.

MALDI-TOF experiments were performed on an Ultraflex TOF-TOF instrument (Bruker) operating in reflector mode and positive polarity. The accelerating voltage was set to 25 kV, the IS 2 was set to 21.9 kV, and the delay time was 20 ns. Calibration was performed using the Bruker Peptide Calibration Standard.

A 1 $\mu$l volume of the peptide solution was mixed with the MALDI matrix (1:1, vol:vol), and the mixture was transferred on a stainless steel target; the droplet was allowed to evaporate before introducing the target into the mass spectrometer.

TOF-TOF mass spectra of the two principal ions (at *m/z* 1910,14 and 1855,11) were recorded using the LIFT device of the instrument. The isolation mass window was set to 1% of parent mass and the laser power boost to induce fragmentation was 80%.

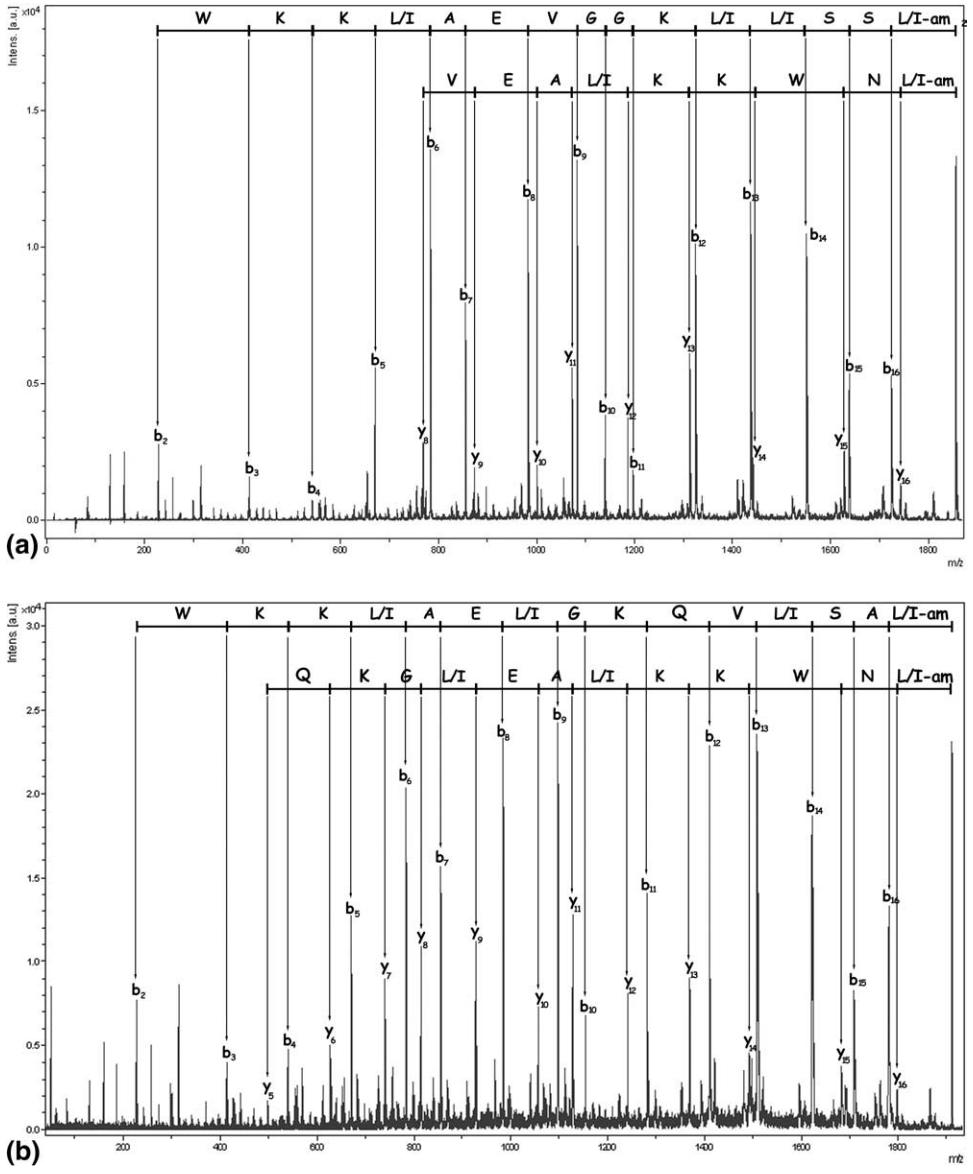


**Figure 2.** MALDI-TOF MSMS spectra of Dominulin A (**a**) and Dominulin B (**b**).

The sequence of each of the two peptides was deduced by interpreting the MS/MS spectra (Figure 2a and b, Table 1 a and b). A modification on the C-terminal peptide was suspected in both the sequences. The presence of a primary amide group, instead of a carboxylic group, was consistent with the experimental data obtained.

MS/MS spectra were also recorded in a LTQ mass spectrometer (Thermo, San Jose, CA). The solution of each peptide was introduced by flow injection mode (5 $\mu$l/min) in the ESI interface of LTQ. The ESI interface and mass spectrometer parameters were the following: I spray voltage 4.2 kV, capillary voltage 35 V, capillary temperature 250 °C, tube lens 180). The MS/MS spectrum was recorded by isolation and collision of the doubly charged ion of each peptide, using an isolation window of 2.0 Th, collision energy of 30% of the total RF energy.

*Peptide sequencing.* The full sequences derived from the interpretation of the MS/MS spectra of the two peptides were compared with the partial sequence obtained by Edman degradation, performed at C.E.I.N.G.E. Laboratory (University of Naples, Italy). This enabled the resolution of uncertainties about the presence of leucine and isoleucine, with the exception of the C-terminal that could have been either an amidate leucine or isoleucine, remained undetermined by Edman degradation. To determine the identity of C-terminal amino acid residue, the two peptides were hydrolyzed in a 1 M HCl solution (100 $\mu$l) for 24 h at 80 °C. The solution was evaporated, reconstituted in 100 $\mu$l of water and then processed using the EzFaast easy-fast amino acid sample testing kit (Phenomenex) for determination of amino acid composition through GCMS. A Hewlett Packard GC-MS system (Palo Alto, CA), composed by a GC 5890 series II coupled with a 5970A MSD operating in EI mode, was used. The ratio

**Table 1.** a) MALDI-TOF MSMS fragment ions of Dominulin A b) MALDI-TOF MSMS fragment ions of Dominulin B

a)

| Dominulin A fragment ions | | | |
|---|---|---|---|
| b Series | | y Series | |
| $b_{16}$ | 1724.91 | $y_{16}$ | 1741.94 |
| $b_{15}$ | 1637.84 | $y_{15}$ | 1627.87 |
| $b_{14}$ | 1550.84 | $y_{14}$ | 1441.82 |
| $b_{13}$ | 1437.73 | $y_{13}$ | 1313.73 |
| $b_{12}$ | 1324.65 | $y_{12}$ | 1185.64 |
| $b_{11}$ | 1196.59 | $y_{11}$ | 1072.56 |
| $b_{10}$ | 1139.52 | $y_{10}$ | 1001.52 |
| $b_{9}$ | 1082.55 | $y_{9}$ | 872.48 |
| $b_{8}$ | 983.48 | $y_{8}$ | 773.41 |
| $b_{7}$ | 854.44 | | |
| $b_{6}$ | 783.40 | | |
| $b_{5}$ | 670.32 | | |
| $b_{4}$ | 542.26 | | |
| $b_{3}$ | 414.12 | | |
| $b_{2}$ | 228.12 | | |

b)

| Dominulin B fragment ions | | | |
|---|---|---|---|
| b Series | | y Series | |
| $b_{16}$ | 1779.92 | $y_{16}$ | 1796.93 |
| $b_{15}$ | 1708.88 | $y_{15}$ | 1682.89 |
| $b_{14}$ | 1621.85 | $y_{14}$ | 1496.81 |
| $b_{13}$ | 1508.77 | $y_{13}$ | 1368.75 |
| $b_{12}$ | 1409.70 | $y_{12}$ | 1240.69 |
| $b_{11}$ | 1281.64 | $y_{11}$ | 1127.61 |
| $b_{10}$ | 1153.58 | $y_{10}$ | 1056.57 |
| $b_{9}$ | 1096.56 | $y_{9}$ | 927.53 |
| $b_{8}$ | 983.48 | $y_{8}$ | 814.45 |
| $b_{7}$ | 854.44 | $y_{7}$ | 757.43 |
| $b_{6}$ | 783.40 | $y_{6}$ | 629.37 |
| $b_{5}$ | 670.32 | $y_{5}$ | 501.31 |
| $b_{4}$ | 542.26 | | |
| $b_{3}$ | 414.20 | | |
| $b_{2}$ | 228.12 | | |

between leucine and isoleucine concentration allowed us to identify the C-terminal amino acid. The assignment was also confirmed by repeating this procedure on the peptides obtained by chemical synthesis (see below).

*Peptide synthesis.* The two main peptides were synthesized by an external laboratory according to the sequence determined as previously described. MALDI-TOF and TOF/TOF spectra of the two synthetic peptides were acquired and compared with those obtained from the natural ones. The purity of the synthetic peptides was determined by RP-HPLC-UV as better than 90%.

*Survey of peptide presence in different colonial components and individual body parts with MALDI-TOF.* Cuticle samples of female and male *Polistes dominulus* were analyzed by MALDI-TOF for the presence of peptides in different ways: (1) the whole body of a single individual was put in 500 μl of methanol for 1 min in a 1.5 ml Eppendorf vial. Water (175 μl) was added to the methanol extract and 5 μl of this solution was mixed with 5 μl of MALDI matrix. One μl of the mixture was dropped on an AnchorChip 600 target (Bruker); (2) a single drop of methanol (about 2 μl) was sprinkled on specific points of the insect body and recollected at once in the same micropipette tip. The drop was then mixed with the matrix directly on the MALDI stainless steel target; (3) anatomical pieces were mounted directly on a MALDI stainless steel target after soaking them with the matrix; (4) the venom apparatus was dissected out of the female abdomen and, by squeezing the venom sac, the venom was collected in a glass capillary and deposited directly on MALDI target where it was immediately mixed with the matrix. In all the procedures, drops of extracts and matrix were allowed to crystallize before introducing the target in the instrument. MALDI-TOF spectra were recorded as previously described.

## Results

Preliminary agar diffusion tests performed with the extract of 10 females in methanol and pentane with *Bacillus subtilis* indicated good antimicrobial activity in methanol extract and a very low activity in pentane extract, compared to the activity of penicillin G, and a null activity of pure solvents. We thus decided to continue our screening with methanol extractions. A methanol extract of the whole body of a single *P. dominulus* wasp was active against *B. subtilis* in agar diffusion tests. Five μl of a 250 μl methanol extract of a single female wasp gave a growth inhibition zone of average diameter of 8.3 mm ($N$ = 10, SD 1.0 mm) in comparison to a growth inhibition zone of 13.3 mm average diameter ($N$ = 10, SD 1.2 mm) given by the penicillin G used as a positive control. In all the 10 tests, 5 μl spots of pure methanol were inactive against the bacterium. HPLC-UV analyses of methanol extract of the whole body of females *P. dominulus* resulted in the separation of 5 main peaks (Figure 1). Peaks 1 and 2 were collected and showed activity against *B. subtilis* in antibiogram agar diffusion tests. Peak numbers 3, 4, and 5, and control pure methanol spots gave only negative results.

HPLC-ESI-MS analyses gave a molecular weight for peaks 1 and 2 of 1854.08 Da and 1909.12 Da., as confirmed by MALDI-TOF analyses performed on the two separated peptides. TOF/TOF spectra put in evidence the peptidic nature of the two molecules (Table 1a and b). They are composed of 17 amino acids and their sequence is INWKKIAEVGGKILSSL for the 1854.11 Da peptide (which we named Dominulin A) and INWKKIAEIGKQVLSAL for the 1909.15 Da peptide (which we named Dominulin B). The sequence was confirmed by Edman degradation performed in an external laboratory; the C-terminal amino acid was determined as an amidated leucine. The spectra of the

**Table 2.** Presence of the two peptides Dominulin A and B in different samples from *P. dominulus* wasps as determined through MALDI analyses

| | | MeOH Micro-extraction of cuticle or of body parts | | Entire body or nest paper MeOH extraction or secretion | |
|---|---|---|---|---|---|
| | Dominulin | Detected | Not detected | Detected | Not detected |
| Young female cuticle | A | | | 10 | 0 |
| | B | | | 10 | 0 |
| Old female cuticle | A | 10 | 3 | 50 | 0 |
| | B | 10 | 3 | 48 | 2 |
| Young males | A | | | 0 | 8 |
| | B | | | 0 | 8 |
| Old males | A | | | 0 | 8 |
| | B | | | 1 | 8 |
| Venom | A | | | 16 | 15 |
| | B | | | 24 | 7 |
| Dufour's gland | A | 3 | 0 | | |
| | B | 3 | 0 | | |
| Eggs | A | 0 | 4 | | |
| | B | 0 | 4 | | |
| Larvae | A | 2 | 12 | 0 | 7 |
| | B | 1 | 13 | 0 | 7 |
| Pupae | A | | | 2 | 4 |
| | B | | | 2 | 4 |
| Nest paper | A | | | 7 | 4 |
| | B | | | 7 | 4 |

synthetic peptides were identical to those of the natural ones.

Antibacterial activity of the two peptides was tested by MIC determination using synthetic samples on *B. subtilis* and *E. coli*. The MIC values on *B. subtilis* and *E. coli* were 2 $\mu$g/ml and 8 $\mu$g/ml, respectively, both for Dominulin A and for Dominulin B.

The survey of the two peptides made with MALDI-TOF on different colonial components (adults, larvae, pupae, nest paper) of various colonies and on different parts of the cuticle and of the body of adult female *P. dominulus* gave the results reported in Table 2. From this, clearly only females produce the two peptides. Females isolated from the nest at the pupal stage presented the peptides when checked three days after emergence from artificial cells (10 out of 10 insects) while males lacked completely the dominulins (8 out of 8 insects) and only one older insect, which lived for a while together with females, presented some traces of the two molecules. On the other hand, clearly, females can apply the peptides on the nest surface as both the dominulins were present in the methanol extracts in 7 out of 11 different nests we analyzed. Pupae presented traces of the peptides only in the 2 specimens (out of 6) that were almost ready to emerge. Larvae ($N$ = 21) presented Dominulin A and B on their cuticle in only 2 cases and 1 case, respectively, while eggs did not present any of the two peptides (4 out of 4 eggs) (see also Figure 3).

Laser shots on specific parts of the cuticle, the antennae and wings, and micro-sampling with methanol on other parts of the cuticle showed that the two peptides are present all over the body of the females (even if sometimes in the same individual they could be detected only in some parts of the cuticle and not in others). The quantities of Dominulin A and B on the cuticle of a single wasp, estimated through the comparison of HPLC-UV signals of wasp methanol extracts ($N$ = 5) and synthetic dominulins, were respectively 7.14 (SD 2.26) $\mu$g and 5.4 (SD 1.99) $\mu$g per wasp.

Dominulins were present in the venom even if not in all specimens examined (16 out of 31 females had Dominulin A, while 24 out of 31 females had Dominulin B), and were present also in the Dufour's gland (an exocrine gland close to the sting apparatus) even though we could analyze only 3 specimens.

## Discussion

Although anecdotal reports about the presence of antibiotic substances on the surface of some insects do exist, only a protein with antibacterial properties, the phenoloxidase, has been reported to be present on the cuticle of several insect species [4]. We found that antimicrobial peptides cover the cuticle of female *Polistes* wasps as a probable first protection against micro-organisms. The two peptides characterized so far (Dominulins A and B) are rich in leucine and show a high degree of identity, especially in the N-terminal part. The MS/MS data also revealed the amidation of the C-terminal, a modification found also in other peptides with antimicrobial activity from insects [27].

The molecular weights of dominulins are similar to those of apidaecins, inducible hemolymph peptides with antibiotic activities reported for several Hy-

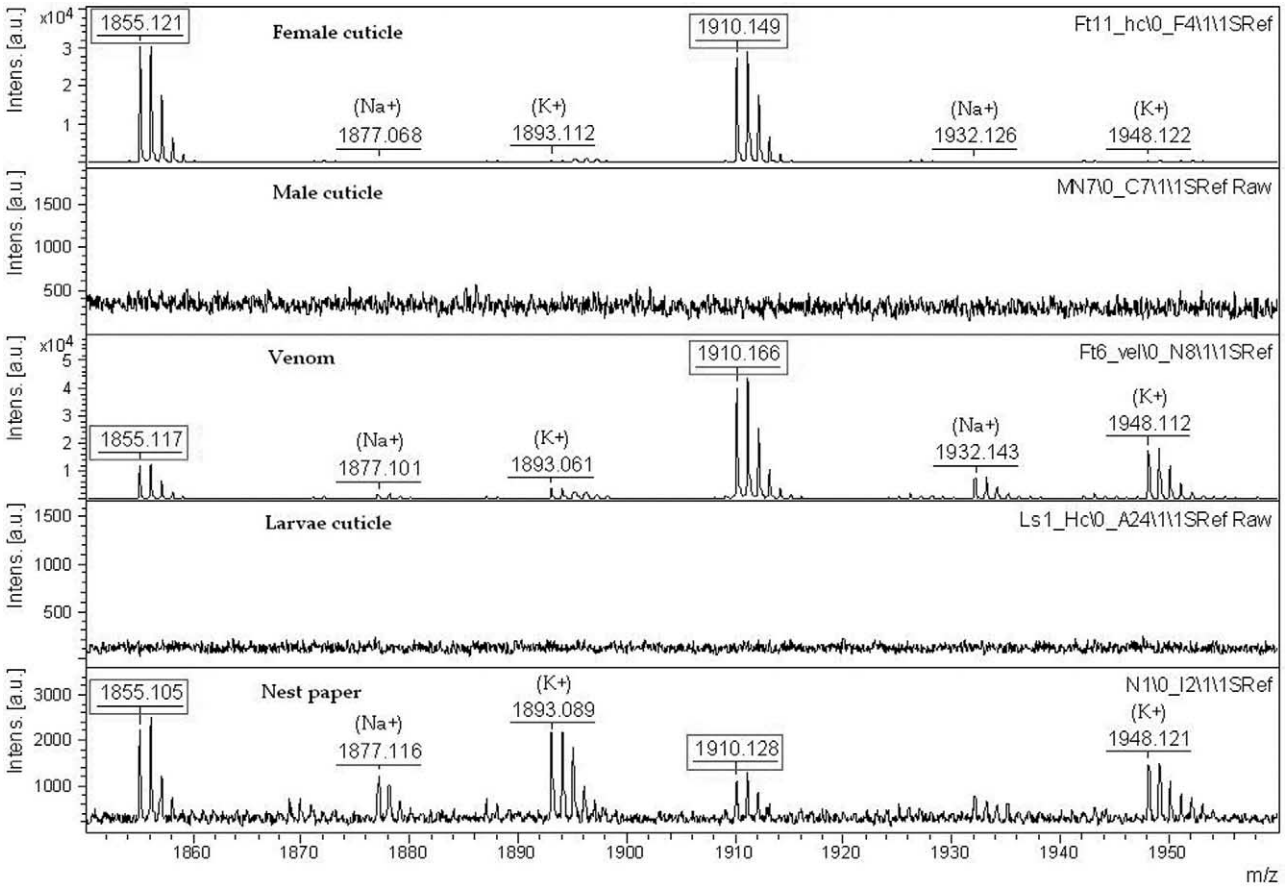


**Figure 3.** Examples of MALDI-TOF spectra in the range 1850–1960 *m/z* of methanol extracts of female and male adults, larvae, venom and nest paper of *Polistes dominulus.*

menopterans [28]. However both the amino acid composition and sequence of apidaecins strongly differ from dominulins.

Between a wide list of antimicrobial and cytolytic peptides reported for the venom of Arthropods [6], dominulins appear more similar to some peptides included in the class of mastoparans, hemolytic tetradecapeptides which have from 7 to 10 hydrophobic amino acid residues and from 2 to 4 lysine residues in their primary sequence. Mastoparans have been found in the venom of various solitary and social wasps [29]. The known mastoparans, which are more similar to the dominulins, are the so-called Mastoparan X from the venom of the vespine *Vespa xanthoptera* [30] (14 amino acids, having the same sequence of the first four residues from the N terminal of the dominulins), the peptide B from the venom of the Polistine wasp *Protonectarina sylveirae* [19] (14 amino acids, having the same sequence of the first four residues from the N terminal of the dominulins) and that from the venom of *Parapolybia indica* [31] (14 amino acids, having the same sequence of the first five residues from the N-terminal of the dominulins). However, the only mastoparans described so far from the venom of a *Polistes* (*P. jadwigae* from Japan), have only a sequence of four amino acids similar to that of Dominulin A (second to sixth from the N-terminal) and two more residues (in Positions 11 and 13) similar to those present in the sequence of Dominulin B [32] (see Table 3). Thus, even if longer than 14 amino acid residues, dominulins could be included in

**Table 3.** Comparison of AA sequence between Dominulins and other mastoparan peptides described from the venom sac of other social wasps

| Peptide name | Social wasp | aa. Sequence | Reference |
|---|---|---|---|
| Dominulin A | *Polistes dominulus* | I N W K K I A E V G G K I L S S L | This paper |
| Dominulin B | *Polistes dominulus* | I N W K K I A E I G K Q V L S A L | This paper |
| Polistes mastoparan | *Polistes jadwigae* | V D W K K I G Q H I K S V L | [32] |
| Mastoparan X | *Vespa xanthoptera* | I N W K G I A A M A K K L L | [30] |
| Peptide B | *Protonectarina sylveirae* | I N W K A L L D A A K K V L | [19] |
| Venom sac peptide | *Parapolybia indica* | I N W K K M A A T A L K M I | [31] |

the mastoparans and the venom could be the source of these peptides, which could be spread all over the cuticle by the frequent grooming movements of the female wasps. It is known that mastoparans injected with the venom can cause hemolysis in mammal cells and mast cell degranulation whilst another important biological activity of these substances is the activation of venom enzymes [29]. Here we observe that another important function of these peptides can be promoting the formation of a cuticular barrier against micro organisms. These substances, moreover, end up constituting a social protection against infections for the whole colony when they are applied to the nest surface. However, uncertainty about the source of these peptides remains, as they were systematically found on the cuticle but not always in the venom, where Dominulin B was more prominent than Dominulin A. This difference could have been caused by errors in the preparation procedure or by other unknown factors (including differential secretory activity of the two substances by the venom glands in different insects and at different ages).

Our microbiological tests have to be considered as preliminary as they were limited to only two bacteria species; however the dominulin MIC values are in the range of MICs (1–8 $\mu$g/ml) of the best natural antibacterial peptides against a wide number of bacteria [33], considering also the 90% purity of our synthetic peptides. For example, MIC values of dominulins on *B. subtilis* and *E. coli* were lower than those of Protonectin (an *Agelaia pallipes* venom peptide with antimicrobial activity) on the same bacteria [18]. At this time, we are enlarging the range of tests against other species of bacteria and fungi, including well known pathogens for insects and man, to verify the effective importance of these compounds in the colonial and individual protection of these wasps.

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